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Research Article

Investigation of using cave bacteria in the production of self-healing mortars

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ABSTRACT

Self-healing concrete is a type of concrete formed by delayed or secondary hydration of cement and its particles with water in the matrix leaking through cracks, and calcium hydroxide and carbon dioxide on the crack surface react with calcium carbonate to provide protection against leakage through cracks. In recent studies, it has been observed that bacteria can self-repair with the production of calcium carbonate-based minerals and this repair provides protection against leakage, pore permeability and protection with increased strength and durability by reducing the infiltration of harmful substances into the internal structure.

In this study, 32 different batches of samples were produced by using *Bacillus thuringiensis, Pseudomonas putida* and *Sphingomonas mucosissima* bacteria in mortar mixtures prepared by traditional methods with 2 different water/cement ratios and 3 different mineral additives. The bacteria used are cave isolates that provide calcium carbonate formation, and these isolates were multiplied in the appropriate growth culture and then added to the prepared mortar samples together with the mixture water. The specimens were cured with tap water for 28 days and then subjected to suction, ultrasonic, flexural and compressive strength tests. The results of the tests revealed a reduction in the porous structure of the specimens containing bacteria, resulting in lower water absorption rates compared to the reference specimens. It was observed that most $CaCO_3$ was produced by Pseudomonas putida bacteria. However, there was no significant difference in compressive and flexural strengths compared to the reference specimens.

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INTRODUCTION

Thanks to the construction sector which develops in direct proportion to the population growth, the production and consumption of the materials such as cement and lime increase [1]. The high quantity of CO_2 emission leads to the global warming which is one of the most crucial environmental issues of our time. Besides, the available efficiency decreases upon degradation of the soil structure, and the

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biodiversity is affected upon that of the microbial, which caters for the substance cycles inside the soil, [2,3]. Due to such disadvantages suffered, it has been aimed at developing an environmentally friendly mode of production in order to avoid the waste formation in cement production and cement-based repair. The research conducted have shown that the cement-based materials such as mortar and concrete can be healed by chemical and biochemical ways as well. To such end, especially in recent years, studies have been being conducted regarding the use of $CaCO_3$ resulting from the biochemical activities of the microorganisms in repair with cement [4,5].

Although a widely used building material because of its production process, raw material availability, and compressive strength, concrete is highly prone to cracking[6]. The crack formation in the concrete structure occurs due to stress and shrinkage reactions in the structures and is a typical feature of durability in many concrete structures. It is not unusual that such crack formations occur at any time during the service life of the concrete. Notwithstanding they do not affect the strength features of the structures in which they occur, such microcracks render the material porous and permeable. Intrusion of aggressive chemicals such as chlorides, sulphates and acids may have long-term effects such as decomposition of concrete matrix and corrosion of the embedded steel reinforcement and may thereby impede the durability of the structures in the long run.

Repair of the cracks in the concrete structures is, on the other hand, an important yet time-consuming and costly task. As the classical methods would bring along a high cost of maintenance and repair and even the impossibility of intervention in certain cases, studies have been undertaken on the concepts of self-healing concrete [7]. It has been found that the most popular concepts include the limitation of the crack width by use of fibres [8], expansion of the cement matrix in contact with water by use of hydrogels[9,10], reinforcement by an active healing agent that is released upon breaking (biomineralization) and the former methods [11,12]

In the self-healing bacterial concretes, the genetical crack healing capacity of the concrete is among the recent studies. The benefit of having the self-healing properties is that the cracks that could occur in the structure of each concrete due to the nature of the latter can be reduced and repaired without the touch of human hands. Study was made in order to extend the life of the concrete structure of any dimension, form or project, and to provide with extra protection each and every component ranging from elements to steel reinforcements. This process can contribute to saving money, help the durability of the structures is maintained for much longer times, and, by having created a much more sustainable product for the concrete industry, efficiently decrease the CO_2 emission [13].

Bacterial healing involves the precipitation of calcium carbonate at the cracks upon direct impact of the bacteria species such as *Bacillus subtilis* on the calcium compound such as calcium lactate or by formation of urea by ureolytic bacteria such as *Bacillus sphaericus* [12,14,15]. Precipitation of calcium carbonate by means of bacteria is in conformity with the concrete and the formation process is environmentally friendly [16]. Therewithal, the reinforcement corrosion risk is reduced thanks to the oxygen produced during the $CaCO_3$ production. Owing to the *Bacillus* species' qualities including the capacity to form spores that make it suitable to be employed as an agent of tolerance to highly alkaline medium and of humidity as well as of self-healing in concrete, it is widely used in research studies as a bio-agent for calcite settling[13,17–19]. An 18% increase in strength was achieved by using bacteria such as Pseudomonas aeruginosa and Bacillus pasteurii in mortar mixtures for self-healing. Jonkers researched in his study the use of bacteria as self-healing agent in the healing of the cracks occurring in the concrete [20]. De Muynck et al demonstrated that the durability of the cement-containing materials can be improved by accumulation of carbonate on the surface by using *Bacillus sphaericus* [21]. Ramachandran et al. reported the use of bacteria for the purpose of enhancing the durability of the concrete so that it can resist the alkali reaction, freeze-thaw attacks, sulphate effect, drying and contraction [22]. Achalet et al. added the *Bacillus sp. CT-5* bacteria to the mix in order to be able to see the impact on the water absorption and compressive strength in the concrete, and found out an increase of up to 36% in the compressive strength, and, as a result of the calcite accumulation, a lower water absorption rate in the microbial specimens compared to the control. And such fact shows that the durability of the construction materials can be increased by using the *Bacillus* species for the production of "microbial concrete" [23].

In a study conducted by Ghosh et at., a mineral settling method was defined which was of microbial basis aimed at increasing the strength in concrete. It was observed an increase of 25% in the compressive strength of the cement mortar by day 28 following the mix water had been added thermophilic and anaerobic bacteria in the range of 10^5 cells/ml. The increase in strength was attributable to the in increase of the filling material inside the pores of the sand-cement matrix[24]. In the study of Schwantes-Cezario et al. conducted using the *Bacillus subtilis* AP91 bacterium in order to observe the variation of the porosity in the mortar specimens, the bacteria were observed to have filled the pores by giving rise to precipitation of $CaCO_3$ [25]. In the study which Bhaskar et al. conducted by using zeolite-immobilized *Sporosarcina urea* and *Sporosarcina pasteurii* bacteria, an abundance of calcite mineral precipitation at the cracks of fibre-reinforced mortar specimens was observed, while recording a decrease in the water and chloride permeability of the specimens, and an increase in their compressive strength [26]. Xu and Wang's study they conducted in order to observe the calcium carbonate precipitation with the alkaliphilic spore-forming bacteria revealed a healing rate of up to 100% in the cracks on the specimens, and increases by 50% and 130% in their watertightness and compressive strengths, respectively [27]. A study conducted by Vashist and Shukla with bacteria of the species *Bacillus subtilis* for the purpose of increasing the durability in the concrete resulted in the observation of increases in the compressive, tensile and flexural strengths in the bacteria-containing specimens in comparison to the reference [28]. In a study conducted by Yılmazer Polat et al., the effects of ureolytic bacteria in geopolymer mortars on the mechanical strength of the mortar were investigated using the bacterial species *Sporosarcina pasteurii*. According to the results of the study, it was observed that the bacteria added to the mortar mixture using appropriate methods had a decreasing effect on water absorption. The compressive strength reached its highest value within the first 7 days and started to decrease after the 28th day, stabilizing at a constant value by the 90th day[29].

In a study on the survivability and self-healing of bacteria in other types of concrete, it was observed that Sporosarcina pasteurii bacteria can repair surface cracks by adding to geopolymer mortar without encapsulation. When the study was examined, it was seen that after the curing of the geopolymer mortar with bacteria, the healing was slower in mortars prepared with endospores obtained from the solid agar surface, although it had fewer voids compared to the mortar without bacteria. It was observed that the addition of bacterial spores to the mortar during the production phase and the improvement of the medium provided more improvement. However, it was observed that the number of voids increased due to the fact that the bacterial spores added to the samples in superficial healing consumed the nutrients in the environment even if they closed the crack and the bacterial spores in the mortar died due to lack of nutrients. In addition to all these, considering the production difficulties of geopolymer mortars and the fact that bacteria can be contaminated very quickly, it is necessary to produce geopolymer mortars in fabricated form[30]. Studies on the use of microbial agents to repair cracks that may occur due to the low tensile strength of recycled aggregate concretes and thus smaller external stresses have shown that cracks can be healed by metabolic precipitation of calcite. However, it has been observed that the survival rate and hence the self-healing rate increases when the bacteria are encapsulated in a suitable carrier rather than directly incorporated into the concrete [17,31–33].

In addition to all these, some studies have shown that manual or external application of bacteria to existing structures does not provide sustainable improvement. For this reason, the use of bacteria as a long-term and embedded self-healing agent in concrete has been investigated. In one study, it was observed that spores of alkali-resistant bacteria of the genus *Bacillus* produced calcium carbonate-based minerals when added to the concrete mix and activated by water ingress through cracks. However, self-healing was limited to a very short period of one to two months due to the direct, unprotected addition of bacterial spores to the mixture [14–16]. Although the life span of bacterial spores is tens of years in the dry state, it is predicted that the embedding of bacterial spores in the concrete matrix and the reduction of their life span to a few months may be due to the continued hydration of the cement and thus the matrix pore diameter becomes much smaller than the bacterial spores [16]. Considering the results of this study, a new study was conducted and bacterial spores and organic mineral compounds were added to the mortar mixture by immobilizing them in expanded clay particles. At the end of the study, it was found that bacterial spores extended the viability period and thus provided self-healing in the matrix. In the light of viability experiments, it was observed that there was no loss of viability even after 6 months [17,20,33,34].

There are not many studies on the impact of the use of bacteria on human health. Studies have shown that bacteria of the genus *Bacillus* do not pose any threat to human health, but there is no information on whether other types of bacteria are pathogenic. Therefore, more studies are needed to determine whether the use of microorganisms is harmful to human health in the long term [35].

In this study, the self-healing potential of mixtures obtained by using three different pozzolanic materials at different water/cement ratios was tried to be determined. On the one hand, the gap-filling abilities of pozzolanic materials were demonstrated in bacteria-free mixtures, while the gaps that pozzolanic materials could not fill were tried to be filled with the $CaCO_3$ production potential of different types of bacteria, which is the original aspect of this study. In this context, in order to demonstrate the effectiveness of bacteria, water absorption and ultrasound transmission rate tests were performed on the prepared mixtures to obtain information about the porosity structure, and compressive and flexural analyzes were performed to determine the strength capacities.

MATERIALS AND METHODS

Materials Used

In the study, fly ash (FA), blast furnace slag and metakaolin were added to the mixture as mineral additives in order to reduce the mass of cement. Fly ash is preferred in the works due to the ease of recycling and low cost of ashes from thermal power plants [36]. It has been observed that it improves concrete density, ultrasonic impact velocity, porosity and mechanical properties in cement mortar mixtures and helps to reduce cement consumption. It also increases its preferability compared to traditional materials due to its low electrical conductivity, thermal conductivity and expansion, fracture and heat capacity [37,38]. Blast furnace slag is a waste material generated during iron production and is preferred due to its effect on strength and durability, thermal cracking and workability properties of concrete [39]. Metakaolin, on the other hand, is obtained by calcining clay mineral at high temperatures and is preferred for its physical and chemical strength and durability improvement, workability and thermal improvement [40].

In the mortar series, bacteria-containing mixing water produced at the laboratory of the Fundamental and Industrial Microbiology Laboratory of the Biology Department of the Faculty of Sciences of the Istanbul University was used along with the cement of type CEM I 42.5 obtained from AKÇANSA Çimento A.Ş., standard RILEM sand, fly ash obtained from the thermal power plant at Çatalağzı/Zonguldak, granulated blast furnace slag obtained from Bolu Çimento Sanayi A.Ş., and metakaolin obtained from KEMS Industrial Company. The physical and chemical properties of the CEM I 42.5 R cement are shown in Table 1, and the chemical properties of the additives in Table 2 and 3.

Table 1. Physical and chemical properties for CEM I 42.5 R

Chemical Properties % Test Method: TS EN 196-2	CEM I 42.5 R	Physical Properties Test Method: TS EN 196-3 and TS EN 196-6	CEM I 42,5 R
MgO	1.38	Specific gravity (g/cm^3)	3.13
SO_3^{-2}	3.39	Setting time (Vicat-min)	Startup: 165 / End: 199
CaO	62.51	Volume expansion soundness (Le Chatelier-mm)	1
Fe_2O_3	3.73	Blaine Specific surface	3300
Al_2O_3	5.20	Residue on 45 mm sieve	4.9
SiO_2 Soluble	19.97	Residue on 90 mm sieve	0.3
Insoluble Residue	1.08		
Unidentified	0.48	Mechanical properties	CEM I 42.5 R
Loss on Ignition	2.06	2 d	32.4
Serbest CaO Free Lime	1.51	7 d	45.5
Cl^-	0.0400	28 d	57.7
Na_2O/K_2O	0.37 / 0.87	* 40x40x160 mm moulds	
		Mix: for specimens prepared by using 1 part cement, 3 parts CEN ref. sand, 0.50 W/C scale	

Table 2. Chemical properties for metakaolin and granulated blast furnace slag

Chemical Properties (%)	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	CaO	MgO	K_2O	Na_2O	B_2O_3	Loss on Ignition
Metakaolin	56.10	40.23	0.85	0.55	0.19	0.16	0.51	0.24	-	1.10
Blast Furnace Slag	40.55	12.83	1.10	0.75	35.58	5.87	0.68	0.79	-	0.03

Table 3. Chemical properties for the fly ash

Chemical Properties (%)	SiO_2	Al_2O_3	Fe_2O_3	SO_3	CaO	MgO	Free CaO	Na_2O	Cl^-	LOI
Fly Ash	54.08	26.08	6.681	0.735	2.002	2.676	0.11	0.79	0.092	1.36

Table 4. Properties of the Bacteria

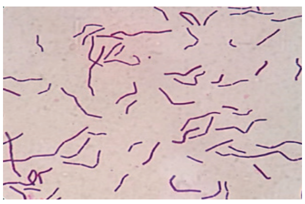
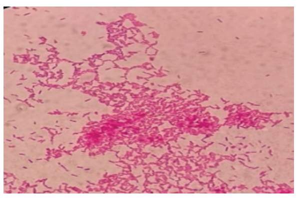
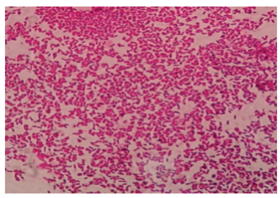
Bacterium used	*Bacillus thrungiensis*	*Pseudomonas putida*	*Sphingomonas mucosissima*
Bacterium Code	Y214	Y234	Y151
Gram Reaction			
Crystal Formation	Positive	Positive	Positive

Figure 1. Bacterial mixture water.

The bacteria used in the mixing water were cave isolates which can precipitate the calcium carbonate. These bacteria isolated from the Yarık Sinkhole cave were identified as *Bacillus thrungiensis*, *Pseudomonas putida* and *Sphingomonas mucosissima* species. None of these bacteria has been encountered in cave microbiological studying literature. Properties of these bacteria are shown in Table 4.

To determine the crystal forming abilities of Y214, Y234 and Y151 coded bacteria, their stock cultures preserved at -86°C were inoculated on sterile TSA (15 g casein peptone, 5 g soy peptone, 5 g NaCl and 15 g agar per liter of distilled water) medium. After 24 hours of incubation at 28°C, a colony from each bacterium was inoculated on 90-mm petri dishes containing sterile B4 medium (2.5 g calcium acetate, 4 g yeast extract, 10 g glucose and 18 g agar per liter of distilled water) and incubated at 28°C. The cultured petri dishes were examined daily under a microscope for crystal formation in the $CaCO_3$ structure. After fixing their ability to precipitate the $CaCO_3$, bacterial suspensions were prepared (approx. 10^9 cells/ml) from a fresh culture on TSA medium and used to prepare the bacteria-containing mixing water (Figure 1).

Production of the Mortar Specimens

The coding used in the production of the mortar specimens is as shown in Table 5. In this coding the first letter denotes the water/cement ratio, the following numbers and the final letters the type of the mineral additive. The mortar mix specimens representing these properties are shown in Table 6.

Table 5. Coding of specimens

W/C	Denomination	Species	Mix Type and Abbreviations
0.5	B	Bacteria-containing	Metakaolin (M)
0.6	C	(1-2-3) reference	Fly ash (U)
		*1: *Bacillus thrungiensis*	Granulated Blast Furnace Slag (O)
		*2: *Pseudomonas putida*	
		*3: *Sphingomonas mucosissima*	

Table 6. Mortar specimens coding and legends

W/C Ratio	Code	Reference (R)	Bacteria		Bacteria Species			Mineral Additives		
			Yes	No	1	2	3	M	O	U
0.5	BR	✓		✓						
	BRO	✓		✓					✓	
	BRM	✓		✓				✓		
	BRU	✓		✓						✓
	B1		✓		✓					
	B1O		✓		✓				✓	
	B1M		✓		✓			✓		
	B1U		✓		✓					✓
	B2		✓			✓				
	B2O		✓			✓			✓	
	B2M		✓			✓		✓		
	B2U		✓			✓				✓
	B3		✓				✓			
	B3O		✓				✓		✓	
	B3M		✓				✓	✓		
	B3U		✓				✓			✓

Table 6. Mortar specimens coding and legends

W/C Ratio	Code	Reference (R)	Bacteria		Bacteria Species			Mineral Additives		
			Yes	No	1	2	3	M	O	U
0.6	CRO	✓		✓					✓	
	CRM	✓		✓				✓		
	CRU	✓		✓						✓
	C1		✓		✓					
	C1O		✓		✓				✓	
	C1M		✓		✓			✓		
	C1U		✓		✓					✓
	C2		✓			✓				
	C2O		✓			✓			✓	
	C2M		✓			✓		✓		
	C2U		✓			✓				✓
	C3		✓				✓			
	C3O		✓				✓		✓	
	C3M		✓				✓	✓		
	C3U		✓				✓			✓

Table 7. Mortar mixing ratios

Mix Code		RILEM Sand (g)	Mixing Water (ml)	Cement (g)	Metakaolin (g)	FA (g)	GBFS (g)
0.5 W/C	W/ additive	1345	225	360			90
	W/ additive	1332	225	360	90		
	W/ additive	1249	225	360		90	
	W/o additive	1350	225	450	0	0	0
0.6 W/C	W/ additive	1346	225	300			75
	W/ additive	1335	225	300	75		
	W/ additive	1332	225	300		75	
	W/o additive	1414	225	375	0	0	0

In the study, 32 series of specimens were produced in total, 8 being reference, and 24 bacteria-containing, using a single type of cement, 2 different water/cement ratios, 3 different types of bacterial strains (*Bacillus thrungiensis, Pseudomonas putida* and *Sphingomonas mucosissima*), and 3 different mineral additives. The fine aggregate granulometry used in the mix was kept constant, and the mineral additive quantity was chosen to be equal to 20% of the cement by weight.

The mortar specimens were prepared in the water/cement ratios of 0.5 and 0.6, and in the mix quantities shown in Table 7, and in the preparation of the mixes, initially the sand, cement and additives were blended in dry condition, then the bacteria were added into the mix with the mixing water. Having been unmoulded, the specimens produced were transferred to and left for 7 and 28 days in the curing conditions. By the end of the 7th and 28th days the specimens were subjected to the water absorption test, UPV test, flexural and compressive strength tests were performed to compare the specimens containing bacteria with those not containing bacteria. For the water absorption test, the specimens of which dry weight measurements had been done before were weighed after they had been removed from the water, and the water absorption ratio was determined by proportioning the difference between their wet and dry weight values to the wet weight value thereof. By this means, the water absorption capacity variations caused in the mortar by the use of bacteria were measured (Figure 2).

Three-point flexural tests were performed on the hardened mortar specimens at 0.005 kN/sec and compressive tests were performed at 2 kN/sec. The test results were measured as per the requirements of the standards ASTM C109 and ASTM C348. At the end of all the tests, the parts of the specimens that had been tested were internally analysed through SEM and XRD analyses.

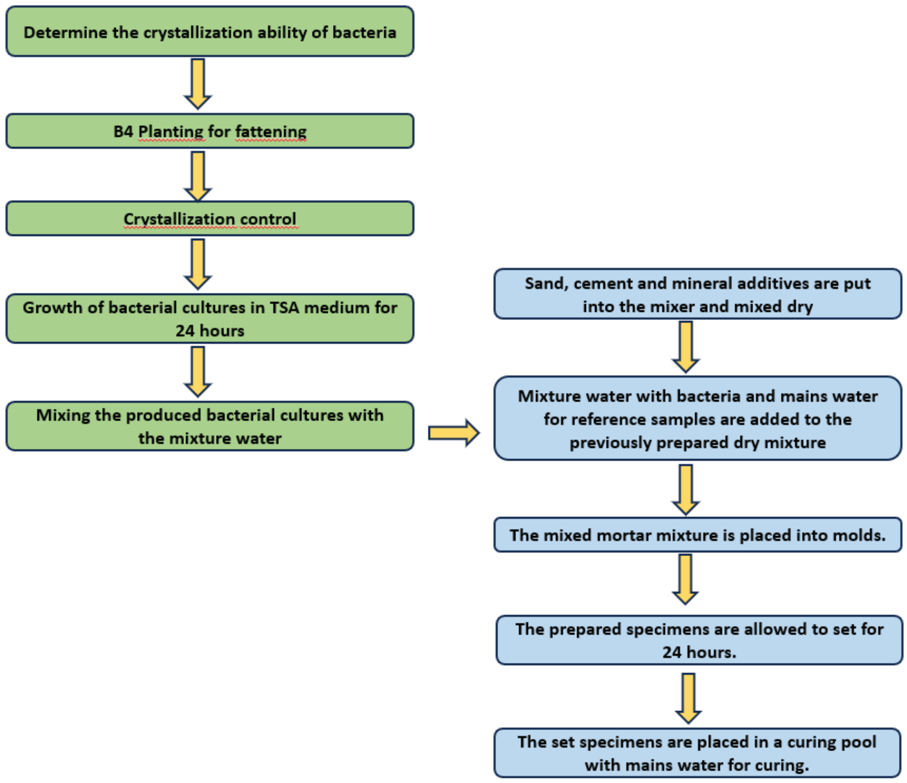


Figure 2. Experimental method.

RESULTS AND DISCUSSION

The results showing the effects of using bacteria in mortar production on the compressive strength, water absorption capacity and ultrasonic permeability of the samples are shown in Figure 3-7.

Water absorption analyses performed at the end of the 28-day curing period revealed a drop in the water absorption capacities in the bacteria-containing mortar specimens in general (Figure 3 and 4). The amount of such drop was found to be 20% in average in the specimens with a w/c ratio of 0.5 compared to the reference specimens, whereas 10% in those with 0.6.

Whereas a result was obtained which was in balance with the bacteria-containing specimens with the w/c ratio of 0.5 according to the UPV test results shown in Figure 3 and 4; the pulse velocity in the specimens with the w/c ratio of 0.6 was observed to have increased compared to the control ones.

Although the porosity had been decreased in the bacteria-added specimens water, and hence the absorption capacities thereof had dropped, no noteworthy positive increase was observed in their flexural and compressive strengths in general terms compared to the control specimens. While some specimens exhibited increased flexural and compressive strengths, some yielded results implying decreases. The review of the three-point flexural tests reveals an apparent increase in the bacteria-added specimens compared to the reference ones. The rate of such increase was measured to be ~7.5% in average in the specimens with the w/c ratio of 0.5, and ~10% in average in those with 0.6. Upon the comparison made between the specimens containing the mineral additives and those not containing the same; a gradual decrease was seen in the strength results, the best strength increase represented by an average rate of 12% in the specimens with the w/c ratio of 0.5 was achieved through fly ash, the one represented by 7% through granulated blast furnace slag, and metakaolin was found to have led to a strength loss of ~5%. Similar results was seen in the specimens with the w/c ratio of 0.6, with the best strength increase represented by an average rate of 20% was achieved through granulated blast furnace slag, the one represented by 11% through fly ash, and metakaolin was found to have let to a strength loss of ~3%.

While an average increase of 15% was observed in a total of 8 specimens for the mortar specimens where granulated blast furnace slag was used, an average decrease of 5% was noted in the remaining 2. The best result was obtained in the specimens with the w/c ratio of 0.5. In terms of compressive strength, on the other hand, an average compressive strength increase of 18% was observed in only 5 specimens, while the remaining exhibited an average decrease of 30% (Figure 5).

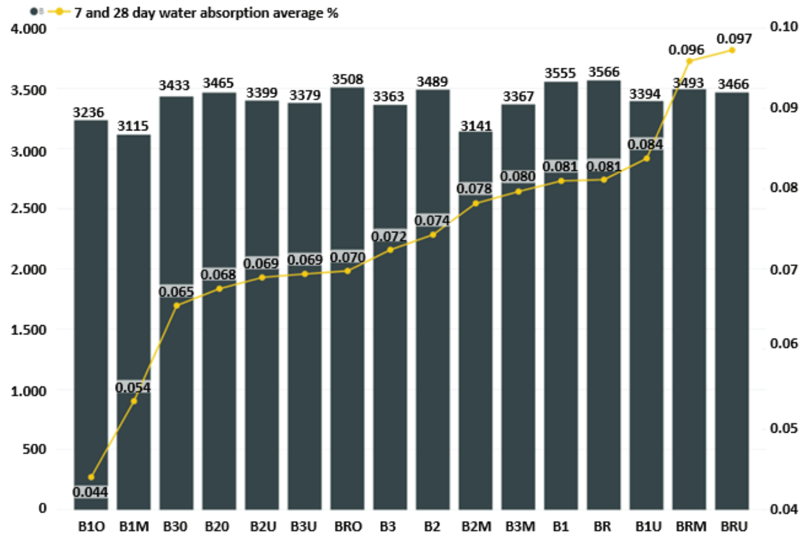


Figure 3. Water absorption and UPV test results of 0.5 w/c.

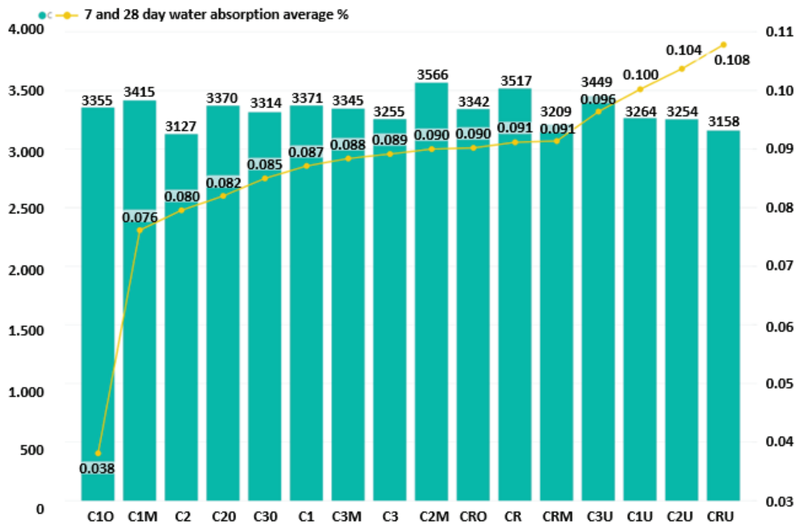


Figure 4. Water absorption –UPV test results of 0.6 w/c.

In the mortar specimens where metakaolin was used no significant positive increase occurred in respect of either compressive strength or water absorption capacity, with an average positive increase of 9% observed in only 5 specimens in total in respect of water absorption capacity reduction, while a decrease of 12% in the others. And an increase of 12% was observed in only 4 specimens in respect of compressive strength, with a decrease of 49% in the remaining ones (Figure 6).

In the mortar specimens where fly ash was used an increase of 8% was observed in total in the water absorption capacity in 4 specimens, whereas a decrease of 13% in the remaining. The study came up with an average increase of 31.5% in terms of compressive strengths in 6 specimens, while an average decrease of 26% in the remaining (Figure 7).

The examination of the specimens produced with a w/c ratio of 0.5 revealed a diminution of the porosity in the bacteria-containing ones compared to the reference ones, and a reduction of the water absorption capacity in comparison to the control specimens accordingly. And it became evident from the examination of the specimens produced with a w/c ratio of 0.6 that their porous structure diminished compared to that of the reference specimens,

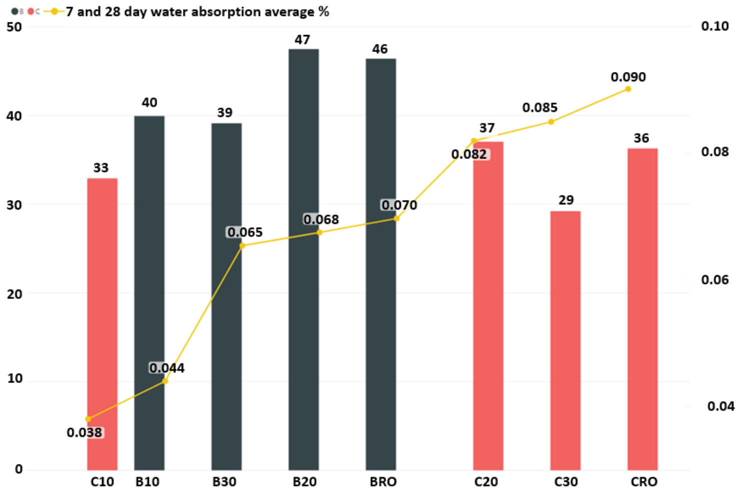


Figure 5. Water absorption-Compressive strength test results of the specimens containing GBFS.

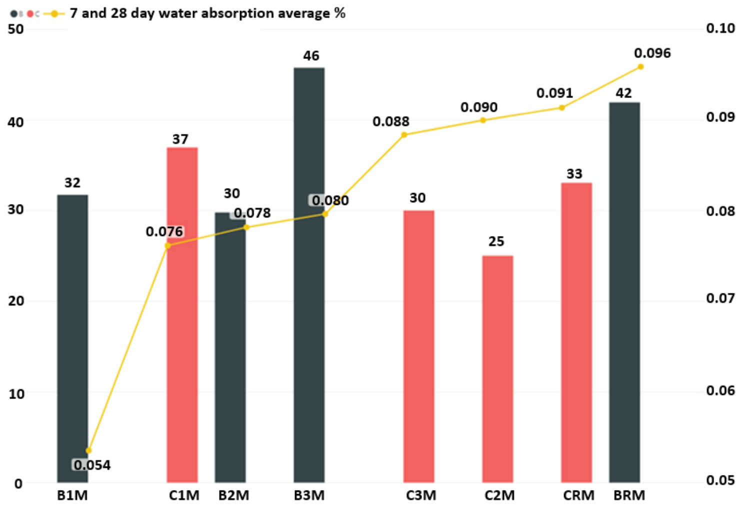


Figure 6. Water absorption-Compressive strength test results of the 0.5-w/c specimens containing metakaolin.

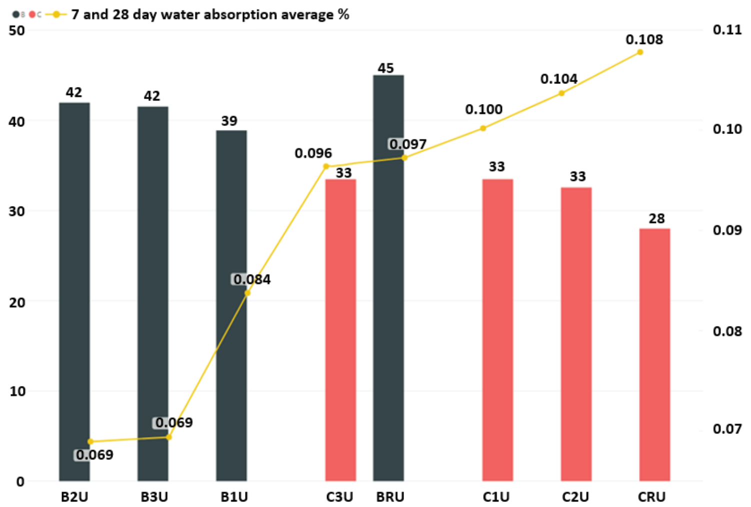


Figure 7. Water absorption-Compressive strength test results of the specimens containing FA.

and a drop occurred in their water absorption capacities accordingly. The biomineralization test performed on mortar specimens using a bacterial strain obtained from the American Type Culture Collection revealed in the specimens a notable extent of $CaCO_3$ increase accompanied by a decrease in the void ratio inside the mortar [41]. It was found in the study conducted by Harshaki et al., on the other hand, that the water absorption ratios were less in quantity in the bacterial cements compared to the reference specimens, and that there had occurred an increase in the concrete compressive strength and hence in the structure's durability associated with the formation of calcites in the fresh concrete pores [42]. In the study of Balam et al. conducted on lightweight aggregate concretes, the use of bacteria allowed the water absorption capacity to be reduced, which in turn led to specimens with a less permeable micro structure [43]. Also, in another study where two bacteria of the species *Sporosarcina pasteurii* and *Bacillus subtilis* were used, it was found that the bacteria species could bring about a reduction of 29-30% in water absorption thanks to the accumulation of calcium carbonate crystals on the surfaces in association with the aggregate porosity [44].

It was found upon the tests performed during the examination of the specimens produced with the w/c ratios of 0.5 and 0.6 that the bacteria-containing specimens yielded better results compared to those of the reference ones. Jafarnia et al. found that calcite precipitation increased the ultrasonic impact velocity, electrical resistivity, compressive and tensile strength of all samples. [45]. It was found in the study of Singh and Gupta that cracking was brought about in the specimens by impregnating the cellulose fibres with the bacteria, and increase was observed in the UPV test results by the end of the healing period. It was found that the crack width of 0.1 to 0.2 had healed by 12.04% in the 14-day curing, and by 15.2% in the 28-day, which ratio was detected to be 8,23% higher than that of the control specimen [46]. The test performed by Kaur et al. using the bacteria of the species *Sporosarcina pasteurii* revealed as a result of the water absorption and UPV tests performed on the specimens that the cracks of an approximate width of 0.6 mm had been closed by the accumulation of $CaCO_3$. The healed specimens exhibited reduction in water absorption capacities, and increase in ultrasonic pulse velocities compared to the control specimens, which was understood to be caused by $CaCO_3$ as evidenced by the SEM and EDS analyses [47]. It has been found out from the studies mentioned herein that calcium carbonate crystals are the substance that is responsible for the reparation by filling the cracks in the process of self-healing, and the results obtained have been seen to support this study.

In line with the results of the compressive strength test performed on the specimens, no increase of compressive strength that could be deemed to be in a notable order was found between the 0.5-w/c-ratio bacteria-containing specimens and the reference ones. The examination of the compressive strength results of the specimens revealed that the maximum compressive strength value obtained was 49 MPa, with the average one being 41.5 MPa. The 0.6-w/c-ratio specimens too exhibited no increase of compressive strength values that could be deemed to be regular in the bacteria-containing specimens compared to the reference ones. The compressive strength results of the specimens showed a maximum compressive strength value of 40.9 MPa, with the average one being 33 MPa. Representing a similar case, a study where Jonkers and Schlangen tested the applicability of bacterial spores for the improvement of the self-healing concrete, could reveal no significant differences between the specimens with and without bacteria as per the results of the tests performed on the specimens produced by using three different species of bacteria in respect of the compressive and tensile strengths, whereas the SEM images revealed precipitation of calcium carbonate crystals [48].

Microstructural Examination

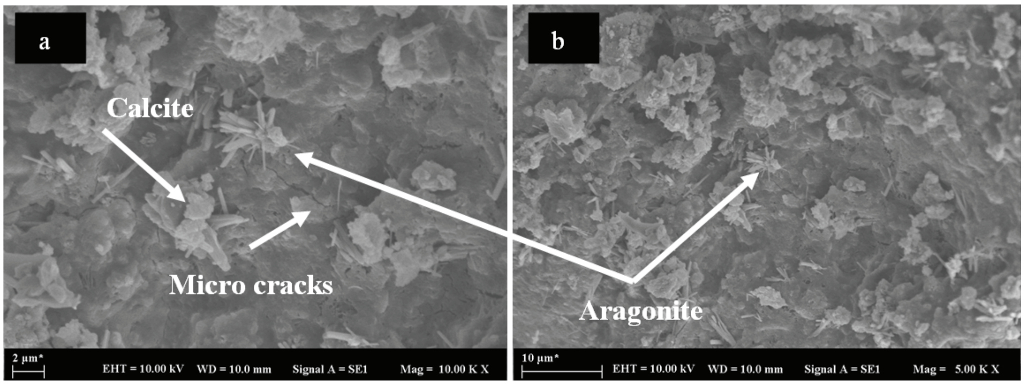
For purposes of imaging of the effect of the bacterial healing inside the mortar, SEM images were taken for the 4 best specimens exhibiting reduction in water absorption capacity. It was detected that the most favourable results in this context were offered by the bacteria *Pseudomonas putida* and *Sphingomonas mucosissima*, whereupon the imaging operations were performed on (B2O, B3, C2) and the control specimen (BRO).

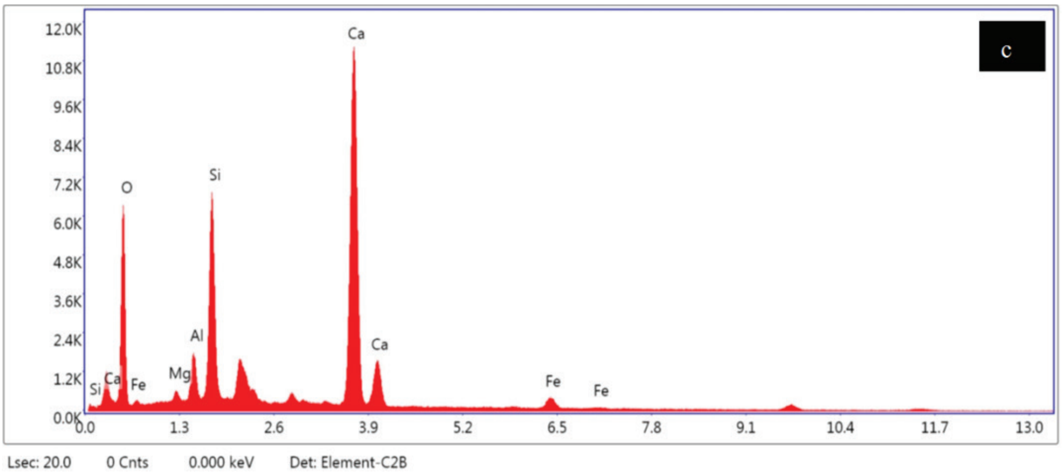
Micrographs (a, b) of the control specimen (BRO) (Figure 8) show both the existence of the C-S-H gel and aragonite, which is a crystal form of CaCO3, as well as non-hydrated slag particles. It is understood that the CaCO3 which formed due to the lack of bacteria in those specimens owes its existence to nothing but hydration. Although the higher percentages of the Ca and O elements detected in the EDS analyses already support the existence of CaCO3, such fact has definitely been detected in the results of the phase analysis.

As shown by the micrographs of the bacteria-containing B3 specimen (Figure9), there is no porous structure left on the inner surfaces of the specimen, as all pores were filled with the C-S-H gels. Calcite and vaterite which are polymorphs of $CaCO_3$ formed by the bacteria are seen in the cells existing inside the structure.

A higher number of porous structures are seen in the micrographs of the bacteria containing B2O specimen (Figure 10), compared to the B3 specimen. Existence of non-hydrated slag particles, C-S-H gel, $CaCO_3$ bridges and vaterite are noted in the specimen's structure.

The micrographs of the bacteria containing C2 specimen (Figure 11) shows that the porous structure was almost fully closed, and vaterite, a polymorph of $CaCO_3$, was formed. A comparison of the bacteria containing B3O, B2O and C2 specimens shows that the best result was obtained from the C2 specimen.





eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
O K	43.92	64.19	2089.31	10.00	0.0707	1.0742	0.1499	1.0000
MgK	0.58	0.56	103.37	12.77	0.0028	0.9953	0.4804	1.0068
AlK	2.66	2.31	594.01	6.43	0.0162	0.9590	0.6256	1.0114
SiK	10.36	8.63	2759.38	4.36	0.0749	0.9805	0.7281	1.0123
CaK	39.68	23.15	6375.63	1.65	0.3630	0.9275	0.9810	1.0051
FeK	2.79	1.17	234.69	7.44	0.0231	0.8283	0.9718	1.0292

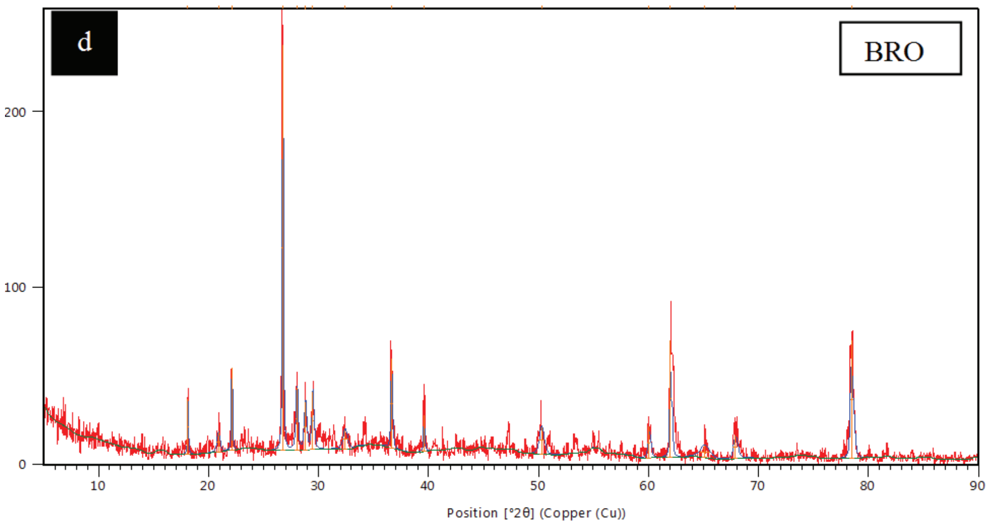
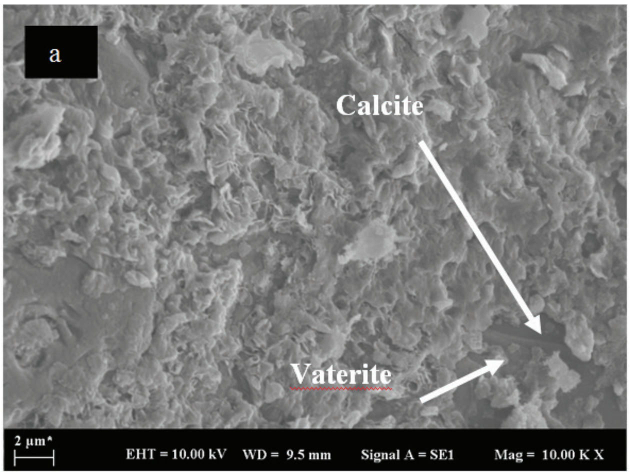
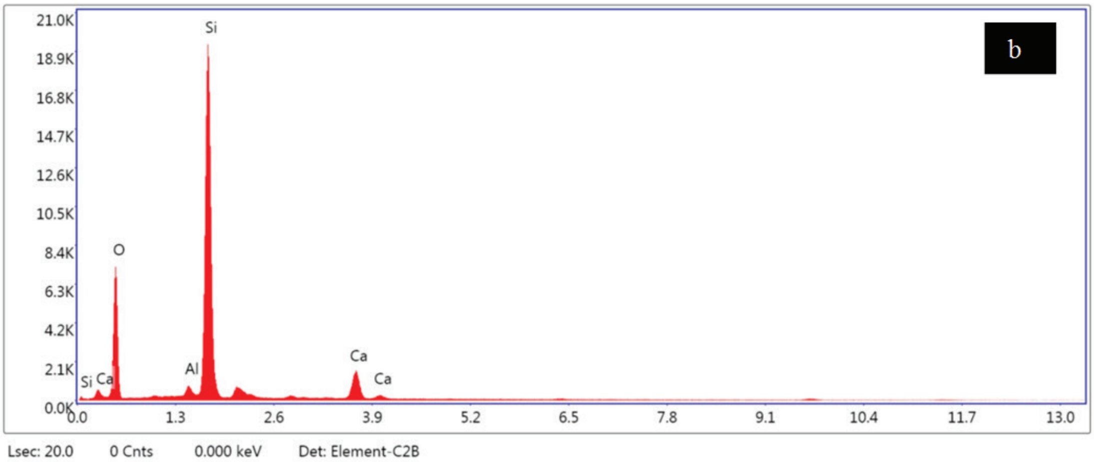


Figure 8. a) 5,000 X SEM **b)** 10,000 X SEM **c)** EDS **d)** XRD Analyses Results in the control specimen (BRO) SEM micrographs.





eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
O K	44.15	59.32	2229.32	8.80	0.1242	1.0553	0.2666	1.0000
AlK	1.45	1.16	233.08	6.96	0.0104	0.9405	0.7413	1.0284
SiK	45.18	34.58	8121.06	2.92	0.3624	0.9613	0.8327	1.0022
CaK	9.21	4.94	835.78	3.33	0.0782	0.9081	0.9278	1.0086

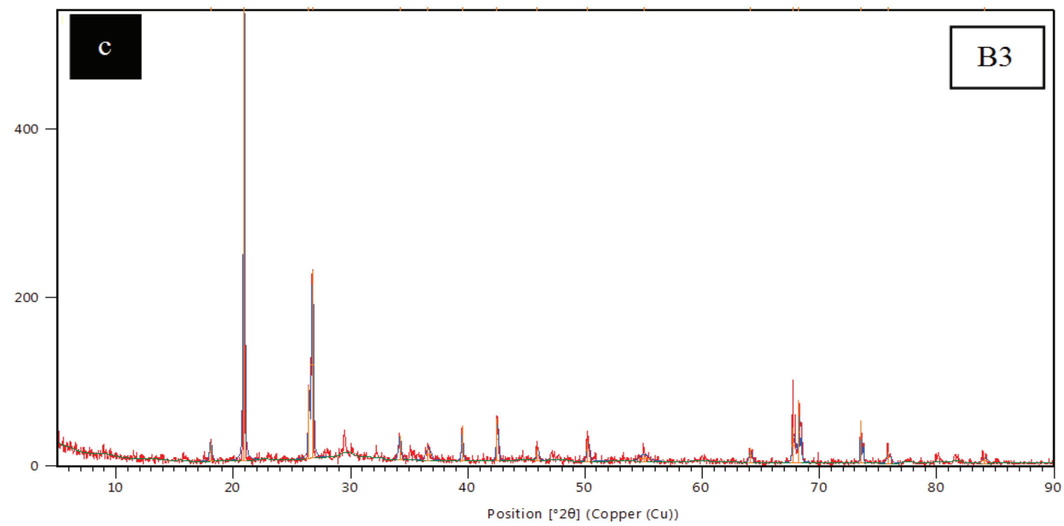
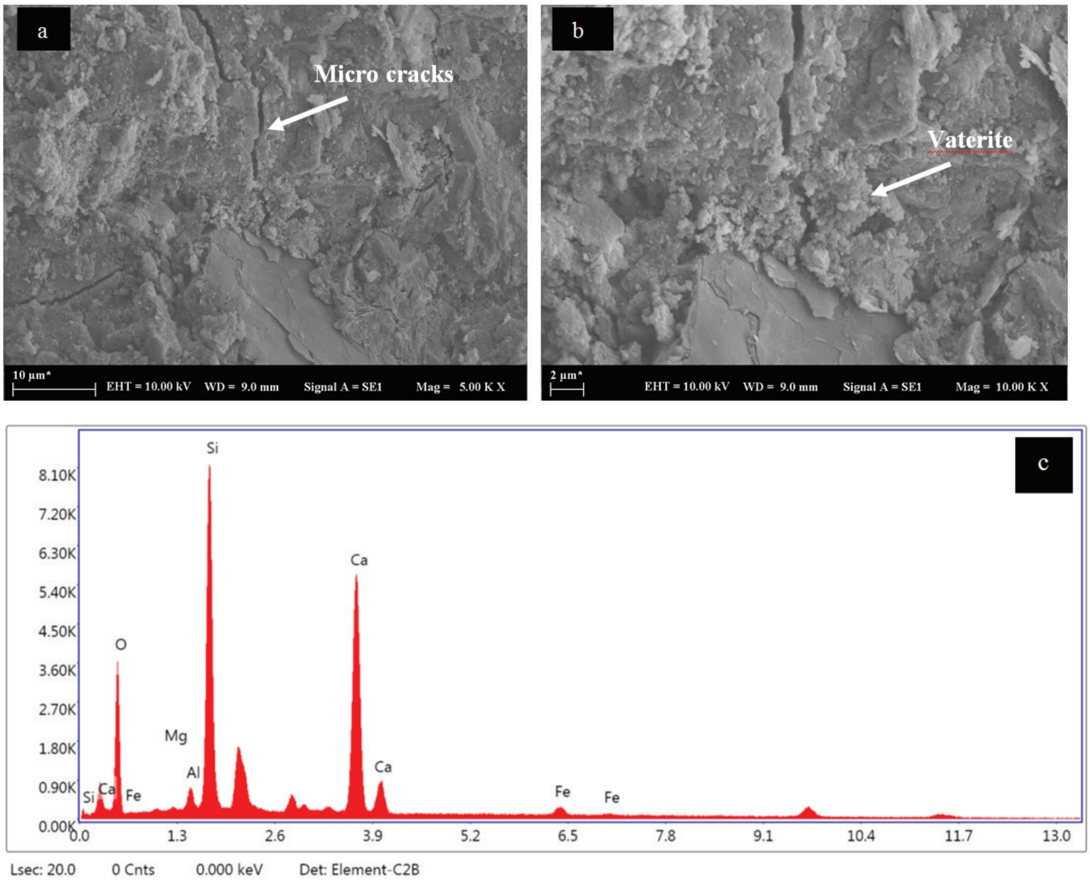


Figure 9. SEM micrograph of the B3 specimen **a)** 10,000 X SEM **b)** EDS **c)** XRD Analysis.



eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
O K	40.37	59.61	1159.35	10.15	0.0674	1.0749	0.1554	1.0000
MgK	0.01	0.01	0.77	99.99	0.0000	0.9959	0.4960	1.0083
AlK	1.13	0.99	153.81	9.19	0.0071	0.9595	0.6461	1.0149
SiK	21.36	17.97	3484.00	3.92	0.1603	0.9810	0.7584	1.0091
CaK	34.34	20.24	3208.68	2.04	0.3087	0.9279	0.9635	1.0057
FeK	2.79	1.18	139.64	10.24	0.0232	0.8286	0.9732	1.0313

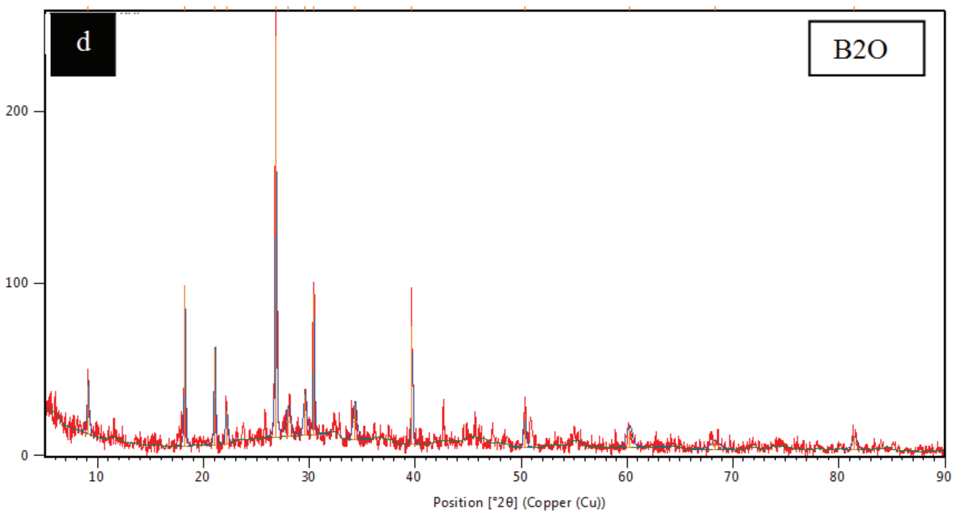
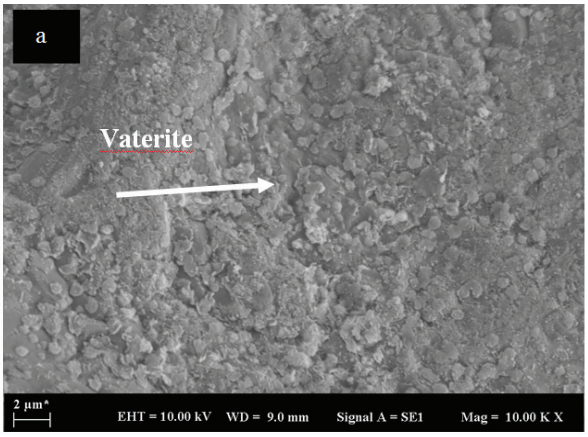
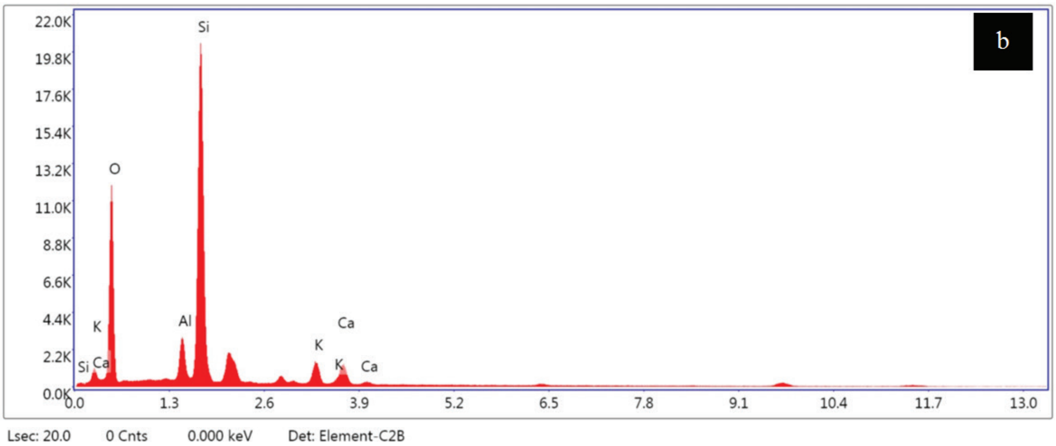


Figure 10. SEM micrographs of the B2O specimen **a)** 5000 X SEM **b)** 10.000 X SEM **c)** EDS **d)** XRD Analysis.





eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
O K	49.43	64.44	3829.86	8.53	0.1469	1.0522	0.2825	1.0000
AlK	5.24	4.05	1183.45	4.83	0.0362	0.9375	0.7226	1.0201
SiK	35.36	26.26	8709.51	3.44	0.2656	0.9582	0.7817	1.0026
K K	4.69	2.50	719.69	4.25	0.0384	0.8888	0.9059	1.0182
CaK	5.28	2.75	695.44	3.84	0.0444	0.9050	0.9201	1.0092

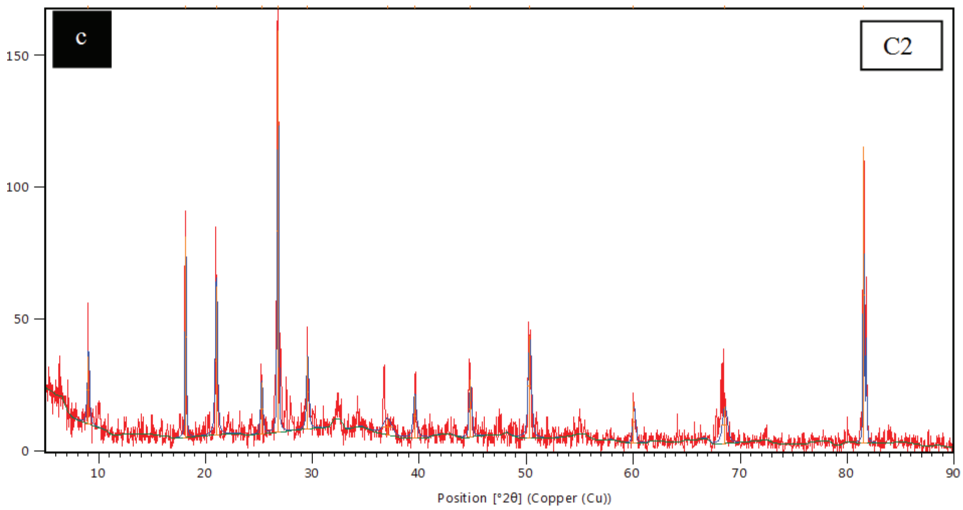


Figure 11. C2 specimen SEM micrographs **a)** 10,000 X SEM b) EDS **c)** XRD Analysis.

CONCLUSION

In the studies carried out to improve the performance of cementitious mortars with *Bacillus* bacteria at a certain concentration, it was determined that the 28-day compressive strength of microbiologically induced mortars increased significantly and this increase was due to the increase in the filling material in the pore after SEM analysis. With the improvement provided by the bacteria, the voids were filled with calcium carbonate precipitation, resulting in a decrease in porosity and preventing possible infiltration, resulting in improvements in concrete in terms of tensile strength at splitting, porosity, acid resistance and chloride resistance as well as compressive strength, resulting in better results compared to conventional concrete [49,50].

In this study were observed the attainable rate of the calcium carbonate precipitation by using bacteria of the species *Bacillus thrungiensis*, accompanied by those of the *Pseudomonas putida* and *Sphingomonas mucosissima*, as well as the effects of the use of bacteria on the porosity of the specimens in the production of mortar. The results obtained from the tests performed revealed that;

- The porous structure diminished through the $CaCO_3$ production in the bacteria-added cement mortar specimens, and accordingly a reduction in the water absorption capacities compared to the reference specimens.
- The bacteria species used are able to perform the expected $CaCO_3$ production in the mortar mix, and the highest rate of $CaCO_3$ production is achieved by the bacteria of the species *Pseudomonas putida*.
- SEM and XRD analyses performed on the specimens by the end of the tests showed that the reduction in the water absorption capacity had its source in the fact that the bacteria had filled the pores by producing $CaCO_3$.
- Due to the decreased quantity of porous structure, a faster sound transmission was detected in the bacteria-containing mortar specimens compared to the control ones, in the ultrasonic pulse velocity tests performed.
- The compressive strength effects of the use of bacteria could not be fully determined as no specific increase or decrease could be noted in the strength.

In the light of these results, addition of bacteria into mortars exert a highly beneficial effect when it comes to mineral-added mortars containing bacteria of different species, considering that such addition lowers the water absorption rates, as well as makes contributions to the durability properties of mortars and concretes. Impermeability of the concrete in particular is highly important as it will prevent the steel reinforcement from corroding, which will then lead to the enhancement of the economic life and the anti-earthquake performance of the buildings.

The crucial role of the durability properties in general terms in reinforced concrete buildings have also been revealed by the studies gaining importance during recent years. Hence, an improvement to occur in the properties of the impermeable concrete, accompanied by the addition of bacteria, is of significance also in respect of the enhancement of the service lives, extension of the economic lives, and increase the seismic performance of the mortars and concretes.

NOMENCLATURE

Al	Aluminum
Al_2O_3	Aluminum oxide
B_2O_3	Boron trioxide
Ca	Calcium
$CaCO_3$	Calcium carbonate
CaO	Calcium oxide
Cl^-	Chloride
CO_2	Carbon dioxide
C-S-H	Calcium-silicate-hydrate
EDS	Energy-dispersive X-ray spectroscopy
FA	Fly ash
Fe	Iron
Fe_2O_3	Iron oxide
GBFS	Granulated Blast Furnace Slag
K	Potassium
K_2O	Potassium oxide
LOI	Loss on ignition
Mg	Magnesium
MgO	Magnesium oxide
Na_2O	Sodium oxide
NaCl	Sodium chloride
O	Oxygen
SEM	Scanning Electron Microscopy
Si	Silicon
SiO_2	Silicon dioxide
SO_3	Sulfur trioxide
$SO_3^{(-2)}$	Sulfite
TiO_2	Titanium dioxide
TSA	Tryptic soy agar
UPV	Ultrasonic pulse velocity
w/c	Water/cement
XRD	X-Ray diffraction

AUTHORSHIP CONTRIBUTIONS

Authors equally contributed to this work.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

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